Summaries of UW–Madison ICTR Novel Methods Pilot Awards, Round 11, 2023

Novel Vitamin A2 Dilution Method for Determining Total Body Vitamin A Status

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Vitamin A deficiency remains a significant global public health issue affecting >200 million children and pregnant women. Risk factors for deficiency include low socioeconomic status, food insecurity, racial/ethnic minority groups, digestive or liver disease, obesity, and bariatric surgery. A challenge of managing vitamin A status is to treat deficiency while avoiding excess intakes leading to toxicity. Clinical guidelines rely on blood retinol or retinol-binding protein (RBP) lab values, which have poor sensitivity to determine vitamin A deficiency or excess as they are homeostatically controlled. 3,4 didehydroretinol (vitamin A2) is a retinol analog that has been used for research and population surveys, but its current application only provides a dichotomous result for vitamin A deficiency and cannot determine vitamin A excess. The proposed method will address these challenges by using vitamin A2 dilution (VA2D) to determine total body vitamin A stores using conventional ultra-performance liquid chromatography (UPLC) and established isotope dilution mathematical models. The primary advantages of the proposed method are increased accuracy to determine vitamin A deficiency or excess and reduced cost and technical requirements for analysis. The new research horizons that this pilot work will enable include translation into humans with powered diagnostic test accuracy studies and clinical trials to evaluating VA2D to improve micronutrient status and health outcomes in line with the NIH vision of precision nutrition.

Engineering Phages for Precision Microbiome Editing

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Collaborators: Phil Huss, UW CALS

Disruption of a healthy gut microbiome, or dysbiosis, is known to cause both acute and chronic illnesses. However, current strategies to neutralize specific microbial threats in the gut microbiome are limited. Here, we outline a proposal for a novel design-build-test-learn (DBTL) method to make precise, programmable edits to the gut microbiome to eliminate dysbiosis-inducing bacteria using engineered bacteriophages (or 'phages'). therapies as well as to assist in elucidating underlying mechanisms involved in human allograft rejection and autoimmunity. Recently, we developed ORACLE (Optimized Recombination, Accumulation and Library Expression), a technique to create large (~106) unbiased libraries of obligate lytic phage variants of any phage gene(s). The development of ORACLE sets the foundation for our proposed DBTL platform. As a proof of concept, we propose to use this method to engineer T7 and K1F phages to precisely eliminate bacteria that produce the genotoxin colibactin, which is strongly associated with the development of colorectal cancer. We will demonstrate the precision of this method in representative human gut microbiome communities. This study is designed to generate a body of knowledge that can be easily leveraged to redesign each phage chassis to different bacterial species, making this process generalizable. We envision this platform as a powerful method to improve preventive and therapeutic options to address bacteria-associated diseases by precision in situ microbiome editing.